FREEZE-DRYING STUDIES WITH STRUCTURAL MUSCLE PROTEINS

by

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Experiences concerning the lyophilizability of the structural muscle proteins are very scarce in literature. Spicer (6) mentions without detailed data that it was possible to lyophilize myosin and actomyosin without loss of enzymatic and "physiological" activity. Mueller (2) states without any reference that "myosin denatures on freeze-drying" in a paper which shows that whole muscles (stripes of rabbit psoas) can be dehydrated without loss of contractility by conventional lyophilization procedures, corroborating thus the finding (3) that a contractile dried muscle can be obtaind by freezing of the muscle and extracting the water by aceton at dry ice temperature. Mainly with practical aims in mind we tried to lyophilize the components of actomyosin, as well as different kinds of actomyosins. In this paper a short description of our results is offered which were, under appropriate conditions, positive.

Methods

Crude actin solutions (1), containing cca. 70% polymerisable protein, myosin (5), myosin-B (7), and myofibrils (4) were prepared by conventional methods. Freeze-drying was carried out with a "Leybold G 08" laboratory apparatus. The material was freezed-in in ampulles of convenient size, in thin layers by the use of aceton dry-ice mixtures. After 5 min.s of evacuation the freezed samples needed no further cooling, since evaporation has kept the material in frozen state. No heating was necessary either to finish the procedure, which took according to the amount of water to be evaporated and the thickness of ice layers 6 – 12 hours. The dried material was kept in a vacuum desiccator with cc. sulphuric acid overnight, and the experiments were carried out on the day following the dehydration procedure.

The dehydrated proteins were redissolved by adding ice-cooled distilled water in amounts equalling the volume of the samples put to dehydration. The redissolved proteins were subjected to enzymatic and viscosimetric tests.

In the case of G-actin the polymerisability, — in the case of F-actin the viscosity, — in the case of actomyosins the ATP-sensitivity and the ATP-ase activity were measured. The two later tests were carried out also on reconstituted actomyosins, in which one of the partners was a redissolved dried preparation.

Viscosity was measured in O s t w a l d viscosimeter at 0° C, or when testing polymerisability of G-actin at 9—10° C. ATP sensitivity was defined as:

$$\frac{\log \eta_{rel} - \log \eta_{rel,\ ATP}}{\log \eta_{rel,\ ATP}} \times 100 \quad (5).$$

ATP-ase of actomyosins was measured in 0.125 M KCl, 4 mM Mg, 4 mM ATP, 0.05 M tris-HCl buffer, pH 7,2 at 20° C; protein concentration 2 mg/ml, incubation time 3 min. In the case of myofibrils the test was the same, but instead of 0.125 M KCl, 0,005 KCl and 0.005 borate-borax buffers were used. The test-solution for myosin was the same as for actomyosin, but contained Ca instead of Mg. The incubation was stopped by the addition of an equal volume of 10% trichloroacetic acid and phosphorus was estimated in an aliquot of the filtrate. Activity is given as μM phosphorus split pro mg protein pro min.

Results

In the following we present the results comparing the characteristics of the lyophilised preparations to the values of the same protein preparations untreated. In table I. the behaviour of actin is shown. As it is seen, the viscosity of lyophilised G-actin shows immediately after redissolution in most cases a significantly higher viscosity than the same G-actin untreated. So lyophilization and redissolution lead to partial polymerisation. It seems thus unpracticable to use lyophilised actin to experiments concerning the polymerisation phenomenon itself. On the other hand, we can get polymerised actin with practically the same viscosity as that of the untreated one, starting with dehydrated G or F-actin. Although lyophilised F-actin gives immediately after redissolution in most cases significantly lower viscosity then the control, on standing at room temperature the viscosity raises to its normal value. Whether the lowered viscosity just after redissolution is the result of a true depolymeri sation caused by the dehydration, or else reflects simply that the complete dissolution of the protein would require more time, we do not know. The solutions of the lyophilised powders were found by visual observation to be quite homogeneous after one to two min. and did flow without any difficulty through the capillary of the viscosimeter.

In Table II. the results of the ATP-ase measurements with lyophilized myosin and actomyosin preparations are presented. All four types of preparations stand dehydration fairly well. The results giving the ATP-sensitivity of the different kinds of actomyosins after dehydration together with results on actomyosins reconstituted from lyophilised components (Table III.), corroborate the general finding, that the proteins concerned can be freeze-dryed without any substantial damage. The last experiments of Table III. show that

Table I.

Viscosity of actin preparations after lyophilisation

The crude actin extract was diluted to 4 mg/ml protein concentration, an aliqot was polymerised by the addition of 0,1 M KCl + 0,001 M $MgCl_2$ at room temperature, for one to two hours. G and F actin samples were put to lyophilisation; the control G and F actins were kept in refrigerator up to the time of experiments (generaly 24 hours). The polymerisation was followed in time at 9 – 10 °C, but we give here only the values after 30 min. and after 24h at room temperature. The values marked "unpolymerised" or for F-actin "immediately after dissolution are viscosities measured immediately after dissolution without any addition. – The viscosities are expressed as logarithms of relative viscosity.

	G – actin					F – actin		
	Control		Lyophilised			Control	Lyophilised	
	Unpolymerised	30 min. polym.	Unpoly-merysed	30 min. polym.	24 hours polym.		Immediately after dissolution	24 hours after dissolution
1.	0,063	0,322	0,063	0,285	0,316	0,324	0,215	0,300
2.	0,074	0,230	0,127	0,214	0,240	0,286	0,162	0,257
3.	0,074	0,230	0,160	0,212	0,236	0,288	0,152	0,260
4.	0,078	0,258	0,116	0,288	0,250	0,260	0,233	0,240
5.	0,097	0,318	0,234	0,320	0,330	0,330	0,312	0,325
6.	0,078	0,281	0,054	0,248	0,260	0,280	0,206	0,280

Table II.

ATP-ase activity of myosin, actomyosin, myosin-B and myofibrils after lyophilisation

Myosin and actomyosins were put to lyophilisation dissolved in 0,5 M KCl; the myofibrils in 0,02 M KCl, 0,02 M borate-borax buffer, pH 7,2; – protein concentration 10 – 20 mg/ml, actin to myosin ratio in actomyosin 1 : 4. For ATP-ase measurement see: "Methods"

		Control	Lyophilised	% activity remaining
Myosins	1.	0,126	0,104	83,0
	2.	0,193	0,145	75,0
	3.	0,131	0,117	89,0
	4.	0,181	0,159	88,0
Actomyosins	1.	0,117	0,105	90,0
	2.	0,163	0,142	87,0
	3.	0,181	0,168	93,0
Myosin-B-s	1.	0,132	0,130	98,5
	2.	0,161	0,152	95,5
	3.	0,197	0,174	88,5
Myofibrils	1.	0,206	0,197	95,5
	2.	0,240	0,214	89,0
	3.	0,250	0,240	96,0
	4.	0,283	0,242	85,5

the unchanged ATP-ase activity of the lyophilized myofibrils results in all probability from the intactness of both main components, as one can extract from lyophilised myofibrils an actomyosin with ATP-sensitivity as high as in the case of untreated myofibrils.

Table III.

ATP-sensitivity of lyophilised actomyosins

The samples were put to freeze-drying in 0,5 M KCl. Actin to myosin ratio in actomyosin 1 : 4. – Actomyosin was extracted from myofibrils with Weber – Edsall solution, for 20 min. precipitated by dilution and redissolved in 0,5 M KCl. – The numbers in parantheses: values in % of control. – For "ATP-sensitivity" see methods.

No. of experiments		1	2	3
Actomyosin	Control	133	108	115
	freeze-dryed	105(79)	75(70)	100(87)
Myosin-B	Control	180	193	165
	freeze-dryed	153(85)	157(87)	138(84)
Actomyosin with lyophilised components	Control	156	170	170
	F-actin freeze-dryed	138(89)	150(88)	156(92)
	Actin freeze-dryed as G and polymerised	134(86)	134(79)	140(82)
	Myosin freeze-dryed	119(76)	121(71)	125(74)
Actomyosin extracted from myofibrils	controll	135	170	–
	freeze-dryed	115(85)	152(90)	–

These experiments show that under the given conditions, the different myofibrillar proteins tolerate lyophilisation well. Surprisingly enough, myosin, actomyosin or myosin-B put to lyophilisation as precipitated gels (in low salt media) were completly inactivated by the procedure. The dried material was found to be completely insoluble. Whether we added 0.5 M (or higher) KCl to the dry powder or we added first distilled water and tried to dissolve the proteins by raising the salt concentration afterwards, we got hardly any protein dissolved and the floccular suspension showed no traces of enzymatic activity.

We gained also some experience regarding the storage of the lyophilised preparations. All the dehydrated proteins were very hygroscopic: kept in a desiccator with calcium chloride they have taken up water as shown by mere inspection, the snow-white powders becoming greyish-yellow in a few days. In a vacuum desiccator with concentrated sulphuric acid they could be kept without apparent change. Nevertheless myosin gradually lost its enzimatic activity. The loss was complete in nine days. F-actin showed in the same intervall only 4% decrease of the viscosity (measured after standing for 24 hours at room temperature in redisolved state). Myofibrils did not show any decrease of activity even if kept for 22 days.

Discussion

The results presented show that the structural proteins investigated can be lyophilised without significant damage. The values of the lyophilised myosin (in per cent of the control) are consequently somewhat lower than the values

for actomyosins. Not enough data are available to decide, whether this differences are significant, but considering that lyophilised myosin becomes progressively inactive, it may be in fact more labile than actin, or actomyosins.

The experience reported may be of practical use in work with muscular proteins. Apart from this, we have some findings which are somewhat unexpected and may have some interest *in se*. It is a general disagreeable experience, that if (by some failure of the refrigerator) myosin or actomyosin solutions or myofibrillar suspensions are slowly freezed-in, there is a complet loss of enzymatic activity. The positive results of the freezedrying experiments show that the inactivation is caused either by the slowness of the freezing-in or by the effect of melting, as a rapid freezing and evaporation of ice had no detrimental effect.

Another finding which has other interest than practical only, is the fact that while myosin and actomyosin *gels* get completly denatured when freeze-dryed, actomyosin of myofibrils sufferes no damage at all under comparable conditions. Clearly, the ordered structure of the myofibrils hinders some sort of interaction between myosin molecules which give on dehydration an irreversible attachment interfering with redissolution. Probably in floccules of precipitated actomyosin some reactive groups become adjacent, which are separated in the ordered actomyosin structure of myofibrils. We hope that by experimenting further on this line, we will be able to get results pertinent to the problem of the disposition of myosin molecules in the myofibril.

Summary

F and G actin, myosin, myosin-B, reconstituted actomyosin *solutions* and myofibril suspensions were subjected to freeze-drying by conventional procedures. In the case of actin the viscosity after polymerisation and the actomyosin formation, – in the case of myosin ATP-ase activity and actomyosin formation, – in the case of actomyosins, ATP-ase activity and ATP-sensitivity, – in the case of myofibrils ATP-ase activity and ATP-sensitivity of actomyosin extracted from it were measured and compared with the respective values of the same protein preparations untreated. The redissolved proteins showed no significant deterioration of the properties investigated.

If myosin or actomyosins were dehydrated as *gels* precipitated by low ionic strength, the material became completely insoluble and lost all enzymatic activity.

Myosin in the dry state lost its activity relatively rapidly (completely in nine days); dehydrated myofibrils could be kept without any deterioration 22 days (longer intervals were not tested).

РЕЗЮМЕ

Растворы F-актина, G-актина, миозина, миозина-B и актомиозина, далее суспенсия миофибриллы были лиофилизированы обычными методами. В случае актина были измерены после полимеризации вискозность и способность образования актомиозина, в случае миозина активность ATP-азы и способность образования актомиозина, в случае актомиозинов активность ATP-азы и чувствительность к ATP в случае миофибриллы активность ATP-азы и чувствительность к ATP извлеченного из ней актомиозина и сравнены с соответствующими особенностями соответствующего необработанного белкового препарата. В исследованных особенностях дегидратированных и перерастворенных белков не было обнаружено никакого значительного ухудшения.

Если миозин или же актомиозин были лиофилизированы при малой ионовой силе в виде выделенного геля, то вещество стало вполне нерастворимым и оно потеряло полностью свою активность в отношении энзимов.

В сухом состоянии миозин постепенно потерял свою активность в отношении к энзимам (через 9 дней полностью); дегидрированная миофибрилла могла быть содержана без всяких потерь в течение 22 дней (более длительное время не было исследовано).

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